

Table 1. Pathogens Isolated and Milk Somatic Cell Counts Pre- and Post-Infusion

Cow No/Qr	Pathogen isolated	Pathogens Day 0	Pathogens post-infusion ⁵	Clinical score [‡] Day 0	SCC post-infusion [*] x 1000/ml	Final SCC ³ x 1000/ml
14LH	<i>S. epidermidis</i>	++++	0	3	2500	237
1850RF	<i>S. aureus</i>	++++	+	4	1890	898
1184RF	<i>S. aureus</i>	+	0	1	531	85
1154LF	<i>Strep. uberis</i>	++++	0	5	2585	333
264LF	<i>Strep. uberis</i>	++++	0	3	3239	269
1176LH	<i>Strep. uberis</i>	++++	++++	2	6354	5992
1163RH	Non-haemolytic <i>E. coli</i>	++++	0	3	846	148
1178LH	No bacteria	0	0	5	1814	90
717RF	No bacteria	0	0	2	933	43

++++ Too numerous to count

* = 500-1000 cfu/ml³

‡ All cows were clinical. The score was evaluated from the clinical appearance of the milk, and any additional abnormalities (eg. visible clots in milk). A value of 0 indicates that the milk was subclinical in which case the SCC was determined. These clinical scores were also used when graphing data.

^{*} Cow 14LH and Cow 717 sampled at day 4, Cow 1184RF and Cow 1154LF at day 6, Cow 302RF at day 7, Cow 1163RH and Cow 1850RF at day 8, Cow 1178LH and Cow 1176LH at day 12 and Cow 264 LF at day 16.

⁵ Cow 1154LF sampled at day 8, Cow 1184RF at day 16, Cow 1163RH at day 25, Cow 264LF at day 35, Cow 14LH and Cow 717RF at day 36, Cow 1850 at day 40 and Cow 1178 at day 55.

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Table 2. Levels of PMN and lymphocytes (CD3) in all udder quarters in Cow 1803 before and after each treatment over the 48-hour trial period.

Quarter	PMN			CD3		
	0hr	24hr	48hr	0hr	24hr	48hr
Right Front (RF) ¹	215	253	101	77.7	218	129
Right Hind (RH) ¹	285	14600	5850	108	2920	4280
Left Front (LF) ¹	551	1330	297	143	596	137
Left Hind (LH) ¹	429	1680	3330	85.1	651	2500

1. Infusion mixtures were prepared as described in Materials and Methods and quarters were treated as follows: RF: untreated; RH: *L.lactis* DPC3147; LF: Antibiotic (Multimast) and LH: Cell-free supernatant.

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Table 3. Somatic cells counts (x 1000/ml) in milk from four udder quarters assayed for immunological responses in Cow 1803.

Days (Hours)	Right Front ^a (RF)	Right Hind ^a (RH)	Left Front ^a (LF)	Left Hind ^a (LH)
0 (0)*	66	1	158	7
1 (24)	149	121	1834	95
2 (48)	43	1150	99	1542
2 (60)	94	Clinical	908	3360
3 (72)	4	2779	69	6296
4 (96)	9	656	81	202
6(120)	69	85	58	54

*day 0= Time of infusion. Samples were taken daily following infusion.

^a RF quarter was left untreated, the RH, LF, and LH quarters were infused with either *L. lactis* DPC3147 overnight culture, the antibiotic Multimast, or cell-free culture supernatant from an overnight culture of *L. lactis* DPC3147.

Table 4. Allocation of treatments amongst teats for trial investigating the ability of dead lactococci to elicit an immune response.

Cow	Quarter	Treatment
1137	RF	Saline ¹
1137	RH	Live ²
1137	LF	Control ³
1137	LH	Dead ⁴
1852	RF	Dead ⁴
1852	RH	Saline ¹
1852	LF	Control ³
1852	LH	Live ²
1570	RF	Control ³
1570	RH	Live ²
1570	LF	Saline ¹
1570	LH	Dead ⁴

1. Saline treatment: Quarters were infused with 2ml sterile saline (0.85% NaCl (w/v)) plus 3ml sterile water for injection.

2. Live culture treatment: Quarters were infused with 2ml overnight broth culture of *L. lactis* DPC3147 plus 3ml sterile water for injection

3. Untreated controls.

4. Dead culture treatment: An overnight broth culture of *L. lactis* DPC3147 was killed by boiling for 10 mins, 2 ml of the dead culture plus 3ml sterile water for injection were then infused into the quarters.

Table 5. Allocation of treatments in each of the twelve quarters of three cows used to determine the effect of infusing different LAB strains on the immune response of cows

Cow #	Quarter	Treatment
1163	RF	<i>Lb. plantarum</i> ¹
	RH	Untreated
	LF	<i>L. lactis</i> 3147 ²
	LH	Bact neg ³
1171	RF	<i>L. lactis</i> 3147 ²
	RH	Bact neg ³
	LF	<i>Lb. plantarum</i> ¹
	LH	Untreated
1181	RF	<i>L. lactis</i> 3147 ²
	RH	<i>Lb. plantarum</i> ¹
	LF	Untreated
	LH	Bact neg ³

1. *Lb. plantarum* treatment: 2ml overnight culture of *Lb. plantarum* DPC4922 and 3ml sterile water for injection.
2. *L. lactis* DPC3147 treatment: 2ml overnight culture of *L. lactis* DPC3147 and 3ml sterile water for injection.
3. Bact neg. treatment: 2ml overnight culture of *L. lactis* DPC5329 (bacteriocin defective mutant of *L. lactis* DPC3147) and 3ml sterile water for injection.

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Table 6. Treatments in each of the twelve quarters of three cows used to determine the effect of infusing different preparations of *L. lactis* DPC3147 on the immune response of cows.

Cow	Quarter	Treatment
275	RF	Freeze dried culture ¹
	RH	Broth culture ²
	LF	Untreated ³
1134	RH	Freeze dried culture ¹
	RF	Broth culture ²
	LF	Untreated ³
2810	LF	Freeze dried culture ¹
	RH	Broth culture ²
	RF	Untreated ³

¹Freeze dried culture: The freeze-dried powder was prepared as described in Materials and Methods and resuspended in a total volume of 5ml.

²Broth Culture: An overnight culture of *L. lactis* DPC3147 was diluted with water ad described in Materials and Methods and used as the infusion mixture.

³Untreated: Untreated controls quarters.

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Table 7. Effects of using *L. lactis* DPC3147 treatments versus treatment with the intra-mammary antibiotic Synulox.

Cow No	Qt	Treatment	Day 0		Day 7		Day 12	
			SCC	<i>S. aureus</i>	SCC	<i>S. aureus</i>	SCC	<i>S. aureus</i>
285	LH	<i>L. lactis</i> 2x24h 5ml	4759	0 ¹	2437	0	6138	0
370	RH	<i>L. lactis</i> 2x24h 5ml	5227	++ ^{1,2}	271	0	2399	0
400	LH	<i>L. lactis</i> 2x24h 5ml	592	++ ^{1,2}	2892	0	1358	0
598	LF	<i>L. lactis</i> 2x24h 5ml	2388	0	3954	0	2697	0
1157	LF	<i>L. lactis</i> 2x24h 5ml	121	0	2094	0	872	0
1170	LF	<i>L. lactis</i> 2x24h 5ml	3690	++	1568	++	3939	++
1183	LH	<i>L. lactis</i> 2x24h 5ml	3638	++	859	0	138	0
1658	RF	<i>L. lactis</i> 2x24h 5ml	5601	+	3143	0	2604	0
1807	LF	<i>L. lactis</i> 2x24h 5ml	390	0	2325	0	1124	++
1827	RH	<i>L. lactis</i> 2x24h 5ml	2892	++	1752	0	2998	+
1867	LH	<i>L. lactis</i> 2x24h 5ml	2057	+++ ¹	1791	+++	2388	+++
1868	LF	<i>L. lactis</i> 2x24h 5ml	661	0	3631	0	1568	0
285	RF	Synulox 3x12h	7659	+	2721	+	5318	+
370	RF	Synulox 3x12h	761	++	209	0	406	0
400	RH	Synulox 3x12h	1735	++	2001	0	1141	0
598	RF	Synulox 3x12h	2160	+++	2073	+++	2862	++
1157	RH	Synulox 3x12h	2999	+	2885	++	1539	+
1157	LH	Synulox 3x12h	86	0	109	0	377	0
1170	LH	Synulox 3x12h	428	0	301	0	854	0
1183	LF	Synulox 3x12h	1602	0	450	0	206	0
1807	RH	Synulox 3x12h	3371	+	3456	0	6052	0
1807	LH	Synulox 3x12h	3030	0	1974	+++	2730	+
1867	RH	Synulox 3x12h	1653	+++	1674	++	1885	++
1868	RF	Synulox 3x12h	6684	+	4051	+	4281	++

1. Bacteria were enumerated and scored according to the following: 0=Absence of pathogens += $<40\text{cfu } 10\mu\text{l}^{-1}$, ++= $40\text{--}400\text{cfu } \mu\text{l}^{-1}$ and +++= $>400\text{cfu } \mu\text{l}^{-1}$

2. *Streptococcus uberis* infection

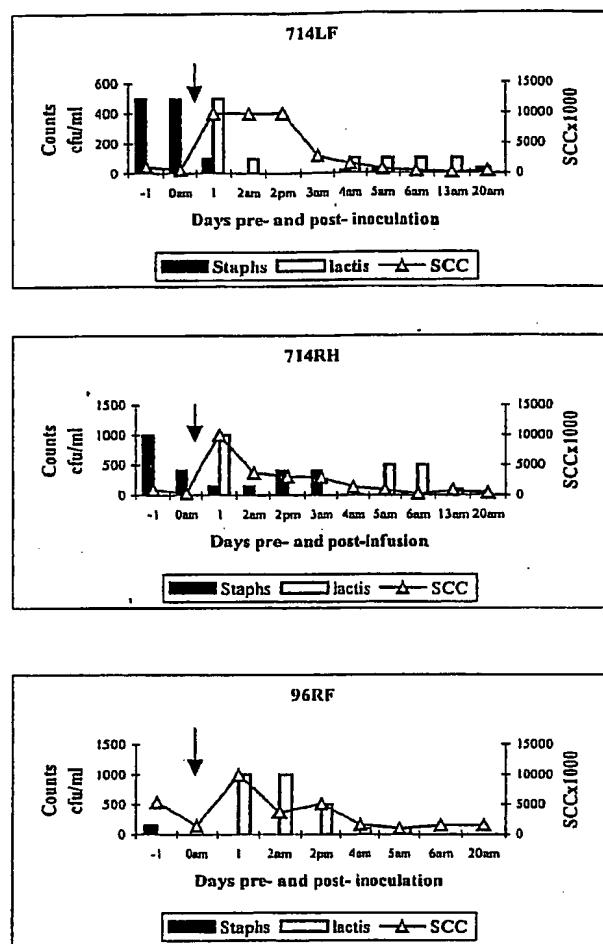


Figure 1A. Somatic Cell Count values and bacterial counts in quarters 714RH, 714LF and 96RF. The black arrow depicts the time of infusion. A clinical response was arbitrarily given a value of $10000 \times 1000 \text{ SCC ml}^{-1}$. Bacterial counts are expressed as cfu ml^{-1} . When less than $400 \text{ bacteria ml}^{-1}$ were present, bacteria were counted precisely. Values greater than this were assigned an arbitrary value of 500 or 1000 (when the bacteria were too numerous to count) bacteria ml^{-1} .

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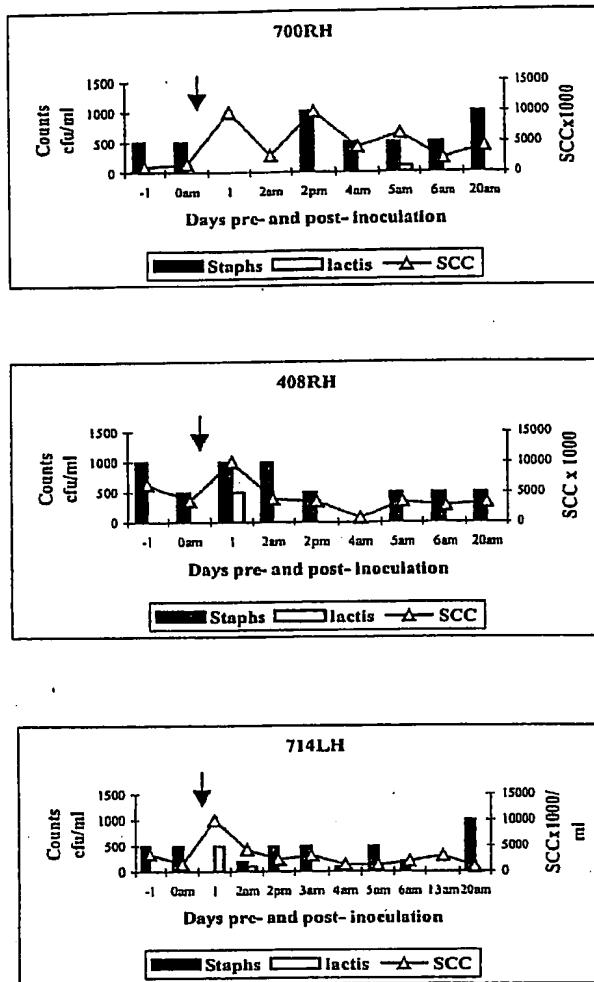


Figure 1B. Somatic Cell Count values and bacterial counts in quarters 700RH, 408RH and 714LH. The black arrow depicts the time of infusion. A clinical response was arbitrarily given a value of $10000 \times 1000 \text{ SCC ml}^{-1}$. Bacterial counts are expressed as cfu ml^{-1} . When less than $400 \text{ bacteria ml}^{-1}$ were present, bacteria were counted precisely. Values greater than this were assigned an arbitrary value of 500 or 1000 (when the bacteria were too numerous to count) bacteria ml^{-1} .

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Cow 1154LF. Milk sampled pre-infusion (left) and post-infusion (right).

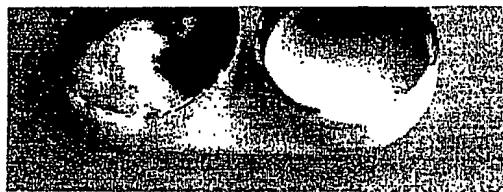


Figure 2A. Appearance of milk from Cow 1154LF sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 7 days post-infusion.

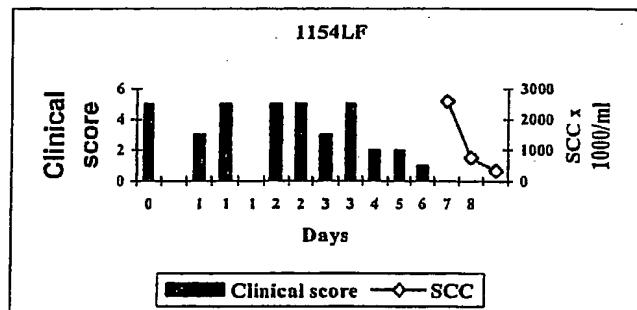


Figure 2B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1154LF sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 1178LH pre- (Day 0) and post- (Day 7) infusion.



Figure 3A. Appearance of milk from Cow 1178LH sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 7 days post-infusion.

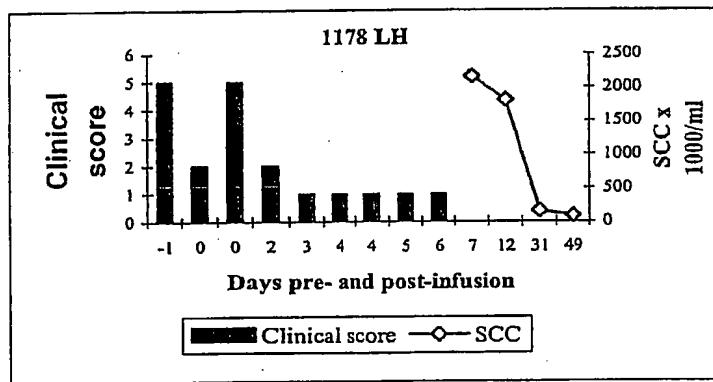


Figure 3B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1178LH sampled pre-and post-infusion of *L. lactis* DPC3147.

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Cow 1850RF pre- (Day 0) and post- (Day 9) infusion.

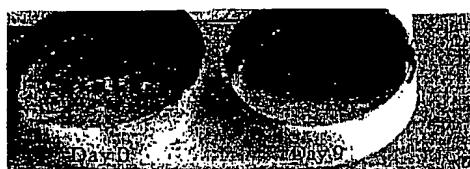


Figure 4A. Appearance of milk from Cow 1850RF sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 9 days post-infusion.

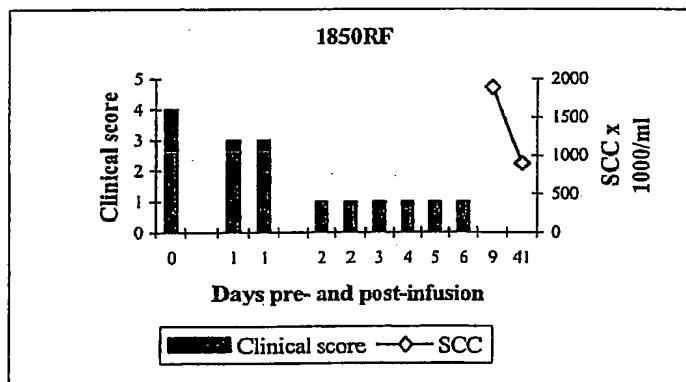


Figure 4B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1850RF sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 1163RH pre- (Day 0) and post- (Day 7) infusion.

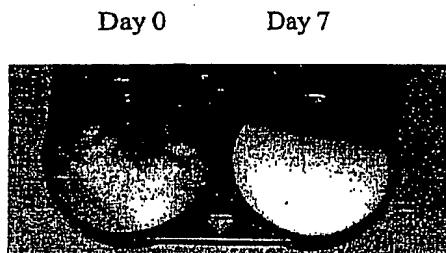


Figure 5A. Appearance of milk from Cow 1163RH sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 7 days post-infusion.

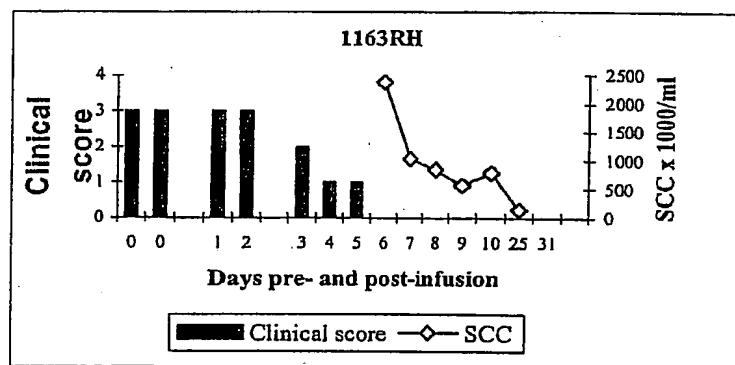


Figure 5B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1163RH sampled pre- and post-infusion of *L. lactis* DPC 3147.

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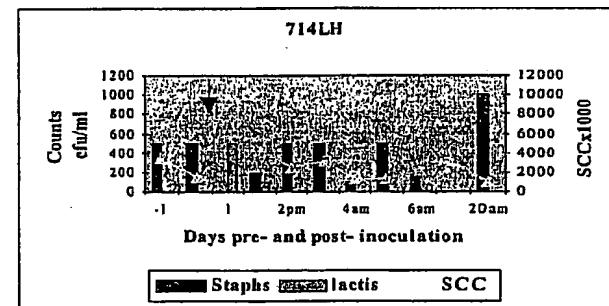
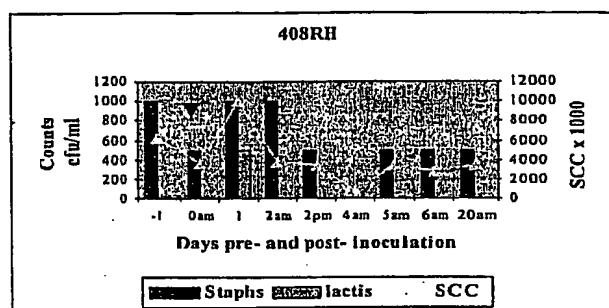
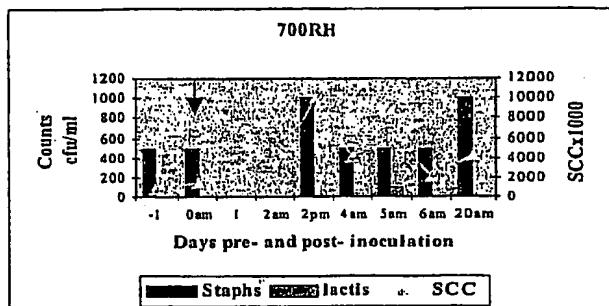


Figure 1B. Somatic Cell Count values and bacterial counts in Quarters 700RH, 408RH and 714LH. The green arrow depicts the time of infusion. A clinical response was arbitrarily given a value of 10000 x 1000 SCC. Bacterial counts are expressed as cfu ml^{-1} . When less than $400 \text{ bacteria ml}^{-1}$ were present, bacteria were counted precisely. Values greater than this were assigned an arbitrary value of 500 or 1000 (when the bacteria were too numerous to count) bacteria ml^{-1} .

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Cow 1154LF. Milk sampled pre-infusion (left) and post-infusion (right).

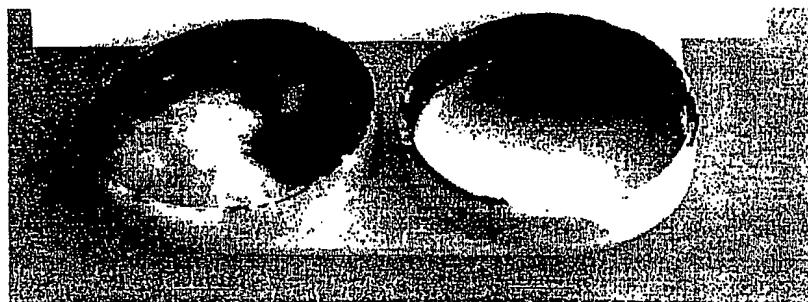


Figure 2A. Appearance of milk from Cow 1154LF sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 7 days post-infusion.

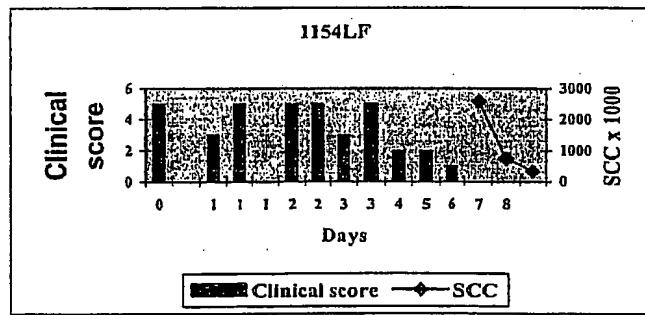


Figure 2B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1154LF sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 1178LH: Milk sampled pre-infusion (left) and post-infusion (right).



Figure 3A. Appearance of milk from Cow 1178LH sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 7 days post-infusion.

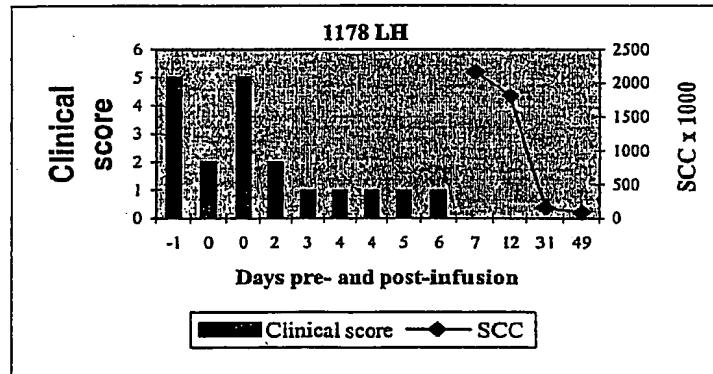


Figure 3B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1178LH sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 1850RF: Milk sampled pre-infusion (left) and post-infusion (right).

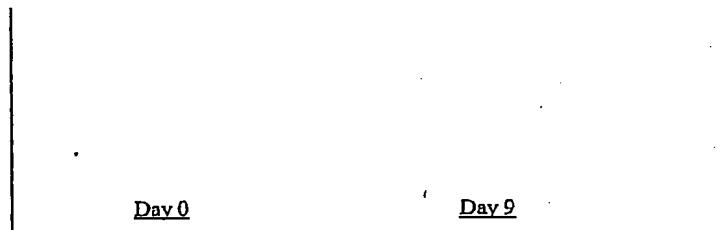


Figure 4A. Appearance of milk from Cow 1850RF sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 9 days post-infusion.

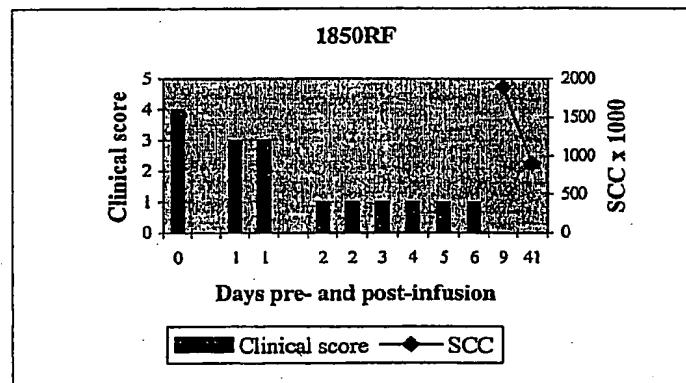


Figure 4B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1850RF sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 1163RH: Milk sampled pre-infusion (left) and post-infusion (right).



Figure 5A. Appearance of milk from Cow 1163RH sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 7 days post-infusion.

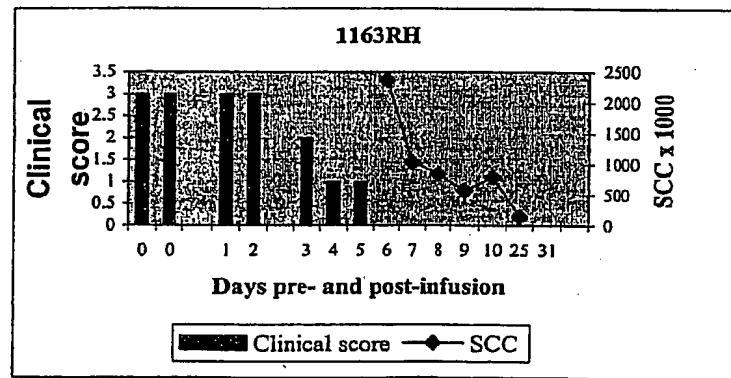


Figure 5B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1163RH sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 1184RF: Milk sampled pre-infusion and post-infusion.



Figure 6A. Appearance of milk from Cow 1184RF sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 3 days post-infusion.

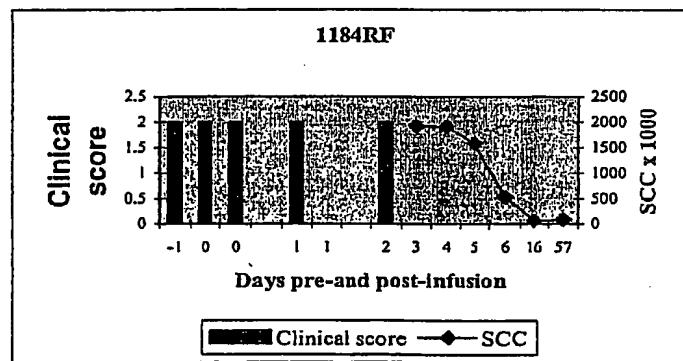


Figure 6B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 1184RF sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 14LH: Milk sampled pre-infusion (left) and post-infusion (right).

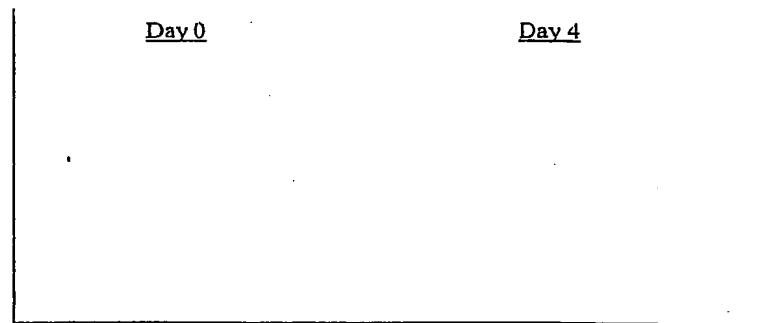


Figure 7A. Appearance of milk from Cow 14LH sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 4 days post-infusion.

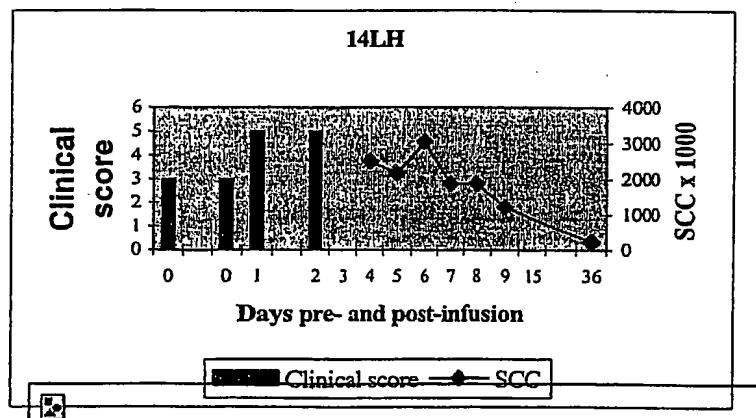


Figure 7B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 14LH sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Figure 8A. Appearance of milk from Cow 717RF sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and 3 days post-infusion.

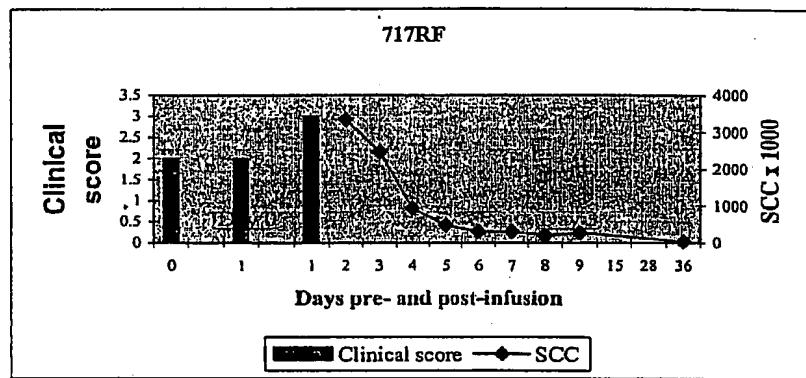


Figure 8B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 717RF sampled pre- and post-infusion of *L. lactis* DPC 3147.

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Cow 264LF: Milk sampled Day 0 (pre-infusion, bottom left), and Days 5, 8 and 12 (post-infusion, bottom right and top left and right, respectively).

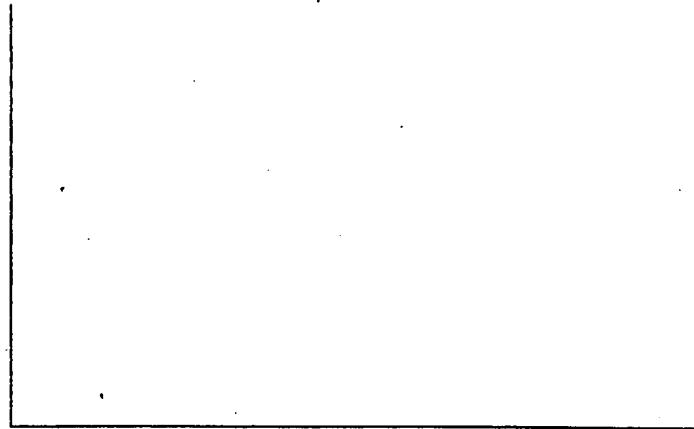


Figure 9A. Appearance of milk from Cow 264LF sampled pre- and post-infusion of *Lactococcus lactis* DPC 3147. Samples shown on Day 0 and Days 5, 8 and 12 post-infusion.

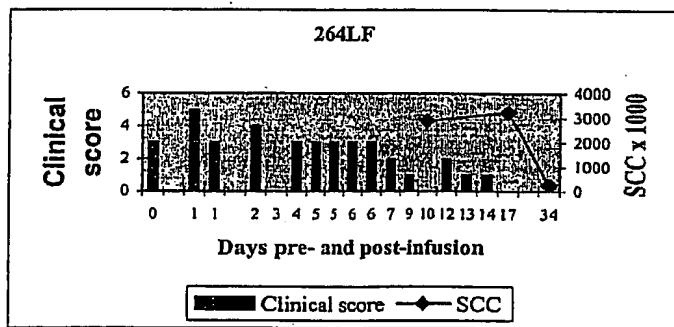


Figure 9B. Graphs of Somatic Cell Count and Clinical Score in milk from Cow 264LF sampled pre- and post-infusion of *L. lactis* DPC 3147.

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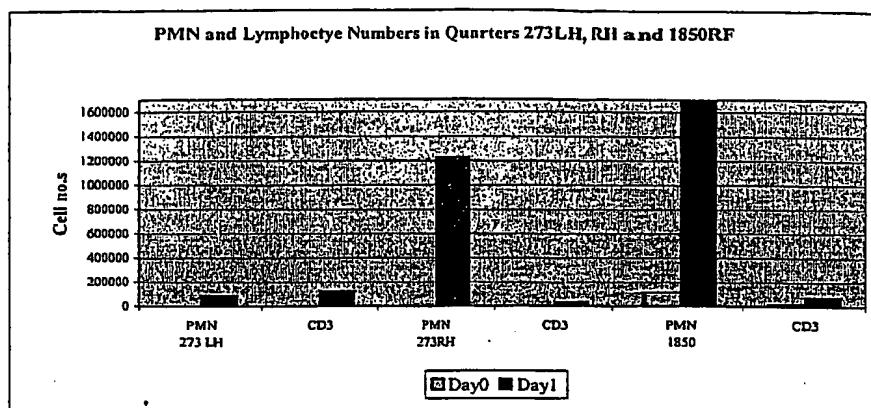


Figure 11. Leukocyte numbers in individual quarters before and after treatment with either *Lactococcus lactis* DPC3147 (Cow 273RH and Cow 1850RF); or sterile broth (Cow 273 LH).

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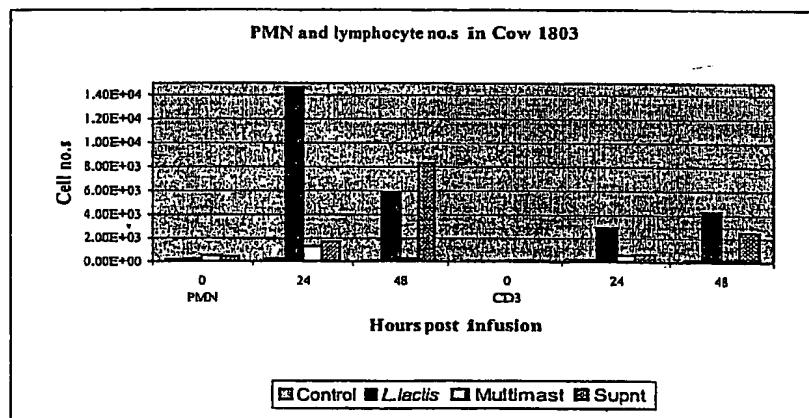


Figure 12. Leukocyte numbers in individual quarters before and after treatment with either *Lactococcus lactis* DPC3147 (RH); antibiotic (LF); cell-free supernatant (LH) or untreated control (RF).

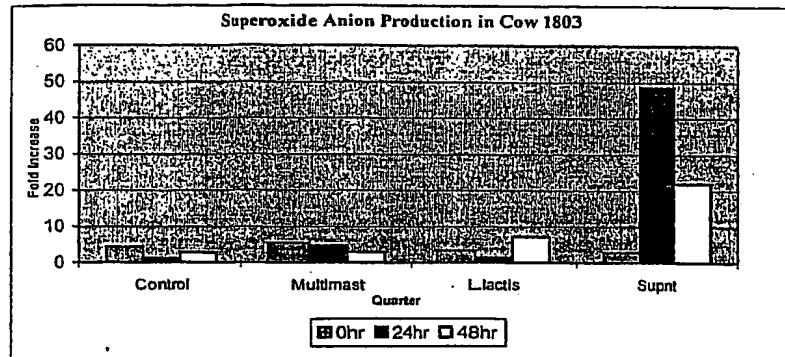


Figure 13A. Superoxide Anion Production by PMN in each of four quarters in one cow (Cow 1803) before and after treatment with either *Lactococcus lactis* DPC3147 (RH); antibiotic (LF); cell-free supernatant (LH) or untreated control (RF).

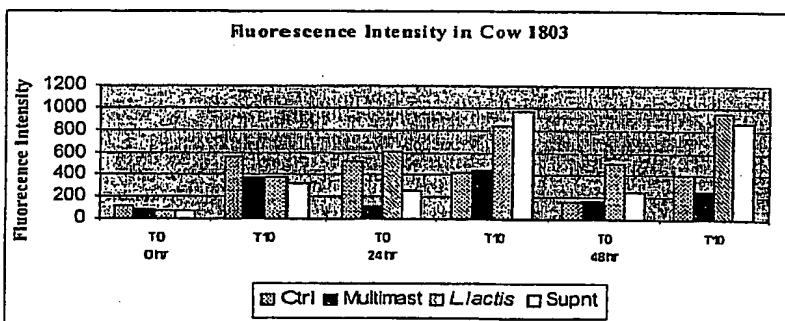
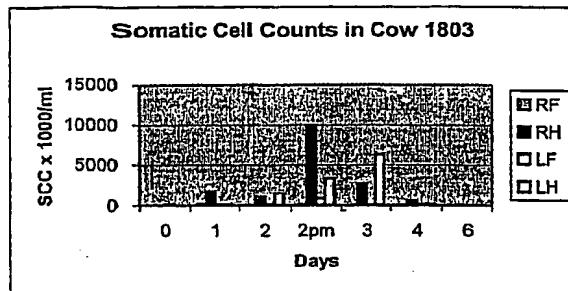


Figure 13B. Levels of superoxide anion fluorescence intensity in each of the four quarters of Cow 1803 before and after treatment with either *Lactococcus lactis* DPC3147 (RH); antibiotic (LF); cell-free supernatant (LH) or untreated control (RF).

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Figure 14. Somatic cell counts in the four quarters of Cow 1803 after infusion with either *Lactococcus lactis* DPC3147 (RH); antibiotic (LF); cell-free supernatant (LH) or untreated control (RF). Day 0 = pre-infusion.



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